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Dear Dr. Franklin:

The reports I have had on your X-ray diffraction work on TMV have interested me greatly. As Jim Watson may have told you, I am measuring the intensity distribution in the equatorial layer line of TMV, using a Geiger counter spectrometer. I understand that you have a plot of the intensity of the equatorial reflections from your photographs, and I would like very much to see your results.

The enclosed graph shows two of the diffraction patterns I have obtained. The geometry of the spectrometer is as follows: four slits with 20 cm between each pair, two for the collimator, and two on the detector arm, with the sample mounted half way between the second and third slits, on the axis of rotation of the spectrometer. Both the collimator and detector paths are evacuated. The slits are all set to the same opening, and slit widths from 0.03 to 0.3 mm are used, depending on the intensity. The geometrical half-width at the detector is thus from 1' to 10'. The slit heights were either 1 cm or .5 cm. Such long slits were required to get enough intensity, with the narrow slits required for high angular resolution. With the longer slits an appreciable fraction of the intensity on the first layer line can be seen by the detector. Most of the data were obtained within 5^{o} of the central beam. At larger angles the intensity was generally too low, and the effect of slit height too pronounced to get significant data. Monochromatization is obtained with balanced Ni - Co foils for Cu Kα radiation. Most of the measurements were made on virus solutions of concentration between 10 and 30 per cent, oriented in capillaries of 0.5 mm diameter.

The lower curve, in the enclosed graph, was obtained using long slits, and the upper one with the short slits. The intensities plotted have been corrected for background scattering, measured with a capillary filled with water. The only significant change in the relative intensity of the subsidiary diffraction maxima on reducing the slit height occurs in the region between 160' and 230', where, with the long slits, an appreciable fraction of the strong reflection on the first layer line in this region is seen by the detector. Even with the shorter slits some intensity from the first layer line can still be seen, as indicated by the shoulder on the fourth subsidiary maximum. Data in the central peak region was obtained with 0.03 and 0.05 mm slits, in the region of the first subsidiary maximum with 0.1 mm slits, and for the rest of the curve with 0.2 and 0.3 mm slits.

The degree to which the minima are filled in is determined primarily by the width of the slits. This slit smearing effect

was calculated for the region of the first zero and maximum of the diffraction pattern of a uniform rod, and was found to be appreciable only for the minimum, where it is the order of that experimentally measured. The value of the intensities at the minima can not be measured with much precision since the counting rates are very close to that of the background and thus the corrected values are sensitive to the accuracy of the correction. Thus the differences in the depth of the minima between the two curves, except for the fourth minimum, are probably not significant. The orientation of the virus samples was checked by birefringence measurements and pin-hole X-ray photographs in some cases. For disorientation to have as great an effect on the diffraction pattern as slit width out to diffraction angles of about 3° a range of orientation angles greater than about 5° from the mean orientation direction would be required.

The theoretical diffraction pattern plotted is the cylindrical average of the square of the structure factor for an hexagonal prism with a thickness, normal to a face, of 152 Å. This is almost identical to the diffraction pattern of cylindrical rod of the same cross-sectional area, that is having a radius of 80 Å. The differences are only noticeable beyond the third subsidiary maximum. No change in the dimension of the uniform rod model would give any better agreement than this. Also there seems to be no way in which the diffraction pattern of a uniform rod could be distorted by slit smearing, sample disorientation, or interparticle interference to give the observed pattern. The interparticle interference from oriented TMV seems to be very similar to that observed in liquids. By extrapolation of the interference effects at small angles, as well as comparison of data taken at different concentrations, it appears that interparticle interference effects in the region of the first subsidiary maximum are not greater than about ten per cent, and are negligable at higher angles.

Assuming that the minima of the diffraction pattern are zeros of the equatorial transform of TMV, and that the sign changes at each zero, at least for the first three minima, the difference between the transform of TMV and that of a uniform rod can be determined. Normalizing to approximately the same zero angle values for the transforms, it seems fairly certain that the difference amplitude is negative for 2θ from zero to about 98', then positive to about 135', and then negative. Beyond about 170' the data is not too reliable and the sign of the fifth maximum is uncertain. The central peak of the transform of TMV is assumed to have the same form as a uniform cylinder of radius which will give the first zero of the transform at the position of the first minimum of the diffraction pattern. If the zero angle normalization is correct, then the difference amplitude can be written as

$$\Delta F = \sum \pm M_i \left\{ J_0(kR_i) - \frac{2J_1(kR_i)}{kR_i} \right\}, \qquad k = \frac{4\pi \sin\theta}{\lambda},$$

Assuming that it is due to a finite number of shells of high and/or

low density. It has been possible to fit the difference amplitude curve by a few terms of this form, the most pronounced being that for a shell of high density at a radius of about 47 Å. The mass coefficient for this term indicates that it may represent the virus nucleic acid. Two other terms with appreciable coefficients represent a low density shell at about 70 Å and a high density shell at about 94 Å. The latter term may be due to interparticle interference effects, but may possibly have some physical significance.

The one conclusion from the results so far that is definite though, is that the density is higher toward the outside of the virus rod, and this high density appears to have its maximum between about 45 and 50 Å. Before carrying out the Fourier-Bessel inversion I would like to try to get better data in the higher angle region and to check the sign assignment. I have prepared oriented TMV in lead acetate solution, and the lead seems to have bound to some extent with the TMV, since the relative intensities of the subsidiary maxima have changed. It is not likely that this is due to a change in the density of the medium since the lead acetate concentration is only 10^{-2}M. I have not yet determined, from the differences between this diffraction pattern and that of TMV in water, where the lead is bound, but it should be possible to do this and to check the signs.

Any details you can send me on your work will be greatly appreciated. I understand you have found that the surface of the virus rod has indentations. Could you let me know what radii you find for the indentation and the projection? I will be leaving Yale at the end of this month for a year at Cal Tech, but if you can write me within the next few weeks would you write me here?

Sincerely yours,

Donald Caspar

Donald Caspar

DC:bh
Encl.